Amendments to the Claims

This listing of claims will replace all prior versions, and listings of claims in the application.

Please cancel claims 2, 3, 8-10, 12, 16, 20, 21, 23, 24, 26-31, 50 and 51.

Please amend claims 1, 6, 13, 14, 15, 18, 19, 25, 27, 32-37, 40, 44, 46, 49, 52 and 56.

Please add new claims 75-88.

1. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising contacting a first linear ds ~~nucleotide sequence~~ nucleic acid molecule, ~~at least~~ a second linear ds ~~nucleotide sequence~~ nucleic acid molecule, wherein said first nucleic acid molecule and said second nucleic acid molecule comprise a topoisomerase recognition site at or near each of their ends, and ~~at least one~~ a topoisomerase ~~in vitro, under conditions such that the topoisomerase covalently links both strands of at least one end of the first ds nucleotide sequence to both strands of at least one end of the second ds nucleotide sequence~~, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ~~ds~~ recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ~~ds nucleotide sequences~~ first and second nucleic acid molecules are joined.

2. (Canceled)

3. (Canceled)

4. (Original) The method of claim 1, wherein the topoisomerase is a site specific topoisomerase.

5. (Original) The method of claim 4, wherein the site specific topoisomerase is a type I topoisomerase.

6. (Currently amended) The method of claim 5, wherein the topoisomerase is a type IB topoisomerase or a catalytic domain of a type IB topoisomerase.

7. (Original) The method of claim 6, wherein the type IB topoisomerase is a poxvirus type IB topoisomerase.

8. (Canceled)

9. (Canceled)

10. (Canceled)

11. (Currently amended) The method of claim ~~10~~ 1, wherein the topoisomerase recognition site is a recognition site for a type IB topoisomerase.

12. (Canceled)

13. (Currently amended) The method of claim 1, wherein one or both of said first ~~ds nucleotide sequence or said at least~~ and said second ~~ds nucleotide sequence or a combination thereof~~ nucleic acid molecules is a polymerase chain reaction (PCR) amplification product produced using a PCR primer pair, wherein ~~at least one~~ each PCR primer of the PCR primer pair comprises a topoisomerase recognition site or a complement thereof~~, thereby producing an amplification product comprising a first end and a second end, wherein at said first end or said~~

~~second end or both, said amplification product comprises a topoisomerase recognition site at or near the 3' terminus~~.

14. (Currently amended) The method of claim 1, further comprising contacting said ~~ds~~ recombinant nucleic acid molecule ~~covalently linked in both strands~~ with an amplification primer pair; and amplifying the ~~ds~~ recombinant nucleic acid molecule.

15. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising contacting a first topoisomerase-charged linear ds ~~nucleotide sequence~~ nucleic acid molecule and ~~at least~~ a second topoisomerase-charged linear ds ~~nucleotide sequence~~ nucleic acid molecule, wherein said first and second nucleic acid molecules are topoisomerase-charged at or near each of their ends ~~in vitro under conditions such that an end of a first ds nucleotide sequence having a topoisomerase covalently bound thereto contacts an end of the at least second ds nucleotide sequence having a topoisomerase covalently bound thereto~~, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ~~ds~~ recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ~~ds nucleotide sequences~~ first and second nucleic acid molecules are joined.

16. (Canceled)

17. (Canceled)

18. (Currently amended) The method of claim 15, further comprising ~~at least~~ a third ds ~~nucleotide sequence~~ nucleic acid molecule.

19. (Currently amended) The method of claim ~~15~~ 18, ~~further comprising at least a~~ wherein said third ~~topoisomerase~~ ds ~~nucleotide sequence~~ nucleic acid molecule is topoisomerase-charged at or near both of its ends.

20. (Canceled)

21. (Canceled)

22. (Original) The method of claim 15, wherein the topoisomerase is a type IB topoisomerase or a catalytic domain of a type IB topoisomerase.

23. (Canceled)

24. (Canceled)

25. (Currently amended) A method of generating a double stranded (ds) recombinant nucleic acid molecule covalently linked in both strands, the method comprising:

a) amplifying a portion of a first ds ~~nucleotide sequence~~ nucleic acid molecule using an amplification primer pair, wherein ~~at least one~~ each PCR primer of the primer pair comprises a topoisomerase recognition site or a complement thereof,

thereby producing an amplified first linear ds ~~nucleotide sequence~~ nucleic acid molecule comprising ~~a first end and a second end, each end comprising a 5' terminus and a 3' terminus~~,

~~wherein at said first end or said second end or at both said first end and said second end, said amplified first ds nucleotide sequence comprises~~ a topoisomerase recognition site at or near ~~at least one terminus~~ both 3' termini or both 5' termini; and

b) contacting the ~~amplified~~ first ~~ds nucleotide sequence~~ nucleic acid molecule; ~~at least~~ a second linear ds ~~nucleotide sequence~~ nucleic acid molecule comprising ~~a first end and a second end, each end comprising a 5' terminus and a 3' terminus, wherein~~ a topoisomerase recognition site at or near ~~said first end or said second end or at~~ each of its ends ~~said first end and said second end, said at least second ds nucleotide sequence comprises a topoisomerase recognition site at or near at least one terminus~~; and ~~at least one~~ a topoisomerase ~~in vitro,~~

~~under conditions such that an end of said amplified first ds nucleotide sequence comprising a topoisomerase recognition site and an end of said at least second ds nucleotide sequence comprising a topoisomerase recognition site are contacted~~, thereby generating a ds recombinant nucleic acid molecule covalently linked in both strands, wherein the ~~ds~~ recombinant nucleic acid molecule does not contain a nick in either strand at the position where the ~~ds nucleotide sequences~~ first and second nucleic acid molecules are joined.

26. (Canceled)

27. (Currently amended) The method of claim ~~26~~ 25, wherein the ~~at least one~~ topoisomerase is a type IB topoisomerase or a catalytic domain of a type IB topoisomerase.

28. (Canceled)

29. (Canceled)

30. (Canceled)

31. (Canceled)

32. (Currently amended) The method of claim 25, further comprising ~~at least~~ a third linear ds ~~nucleotide sequence~~ nucleic acid molecule.

33. (Currently amended) The method of claim ~~25~~ 32, ~~further comprising at least a~~ wherein said third ~~ds nucleotide sequence~~ nucleic acid molecule ~~comprising a first end and a second end, each end comprising a 5' terminus and a 3' terminus, wherein said at least third ds nucleotide sequence~~ comprises a topoisomerase recognition site at or near ~~at least one of said 5' terminus or said 3' terminus~~ both of its ends.

34. (Currently amended) The method of claim ~~33~~ 25, wherein ~~each of~~ said ~~amplified~~ first ~~ds nucleotide sequence~~ nucleic acid molecule and said second ~~ds nucleotide sequence~~ nucleic acid molecule ~~and at least third ds nucleotide sequence comprises~~ each comprise an overhanging sequence at an end comprising the topoisomerase recognition site.

35. (Currently amended) The method of claim 34, wherein the topoisomerase recognition site is at or near ~~at least one 3' terminus~~ both 3' termini, and wherein the overhanging sequence is a 5' overhanging sequence.

36. (Currently amended) The method of claim 34, wherein the overhanging sequences of said ends of said ~~ds nucleotide sequences~~ nucleic acid molecules to be covalently linked are complementary.

37. (Currently amended) The method of claim 25, wherein the ~~at least~~ second ~~ds nucleotide sequence~~ nucleic acid molecule comprises or encodes a regulatory element.

38. (Original) The method of claim 37, wherein the regulatory element is a promoter, an enhancer, a silencer, a translation start site, or a polyadenylation signal.

39. (Original) The method of claim 37, wherein the regulatory element is an initiator methionine codon or a STOP codon.

40. (Currently amended) The method of claim 25, wherein the first ~~ds nucleotide sequence~~ nucleic acid molecule comprises an expressible nucleotide sequence.

41. (Original) The method of claim 40, wherein the expressible nucleotide sequence encodes a polypeptide.

42. (Original) The method of claim 41, wherein the expressible nucleotide sequence comprises an antisense nucleotide sequence, a tRNA, a ribozyme, an RNAi nucleotide sequence, or a triplexing nucleotide sequence.

43. (Original) The method of claim 42, wherein the tRNA is a suppressor tRNA.

44. (Currently amended) The method of claim 25, wherein the ~~at least~~ second ~~ds nucleotide sequence~~ nucleic acid molecule comprises or encodes a detectable label.

45. (Original) The method of claim 44, wherein the detectable label is an enzyme, a substrate for an enzyme, a fluorescent compound, a luminescent compound, a chemiluminescent compound, a radionuclide, a paramagnetic compound, or biotin.

46. (Currently amended) The method of claim 25, wherein the ~~at least~~ second ~~ds nucleotide sequence~~ nucleic acid molecule comprises or encodes a tag.

47. (Original) The method of claim 46, wherein the tag is an oligonucleotide tag or a peptide tag.

48. (Original) The method of claim 47, wherein the peptide tag is a polyhistidine tag, a V5 epitope, or a myc epitope.

49. (Currently amended) The method of claim 25, wherein the ~~at least~~ second ~~ds nucleotide sequence~~ nucleic acid molecule encodes a transcription activation domain or a DNA binding domain.

50. (Canceled)

51. (Canceled)

52. (Currently amended) The method of claim 25, wherein the first ~~ds nucleotide sequence~~ nucleic acid molecule and ~~at least~~ the second ~~ds nucleotide sequence~~ nucleic acid molecule are covalently linked in a predetermined directional orientation.

53. (Original) The method of claim 25, further comprising performing a coupled transcription/translation reaction using the ds recombinant nucleic acid molecule.

54. (Original) The method of claim 25, further comprising transfecting a cell with the ds recombinant nucleic acid molecule.

55. (Original) The method of claim 25, wherein a primer of the primer pair comprises a complement of a type IB topoisomerase recognition site, said amplification primer further comprising a 5' hydroxyl group.

56. (Currently amended) The method of claim 25, wherein the primer comprising the topoisomerase recognition site or complement thereof further comprises ~~a nucleotide sequence~~ of ~~about~~ 2 to 12 nucleotides 5' to said topoisomerase recognition site or complement thereof.

57 to 74. (Cancelled)

75. (New) The method of claims 1 or 13, wherein said first and second nucleic acid molecules comprise a topoisomerase recognition site at or near each of their 3' termini.

76. (New) The method of claim 15, wherein said first and second nucleic acid molecules are topoisomerase-charged at or near each of their 3' termini.

77. (New) The method of claim 25, wherein said first and second nucleic acid molecules comprise a topoisomerase recognition site at or near each of their 3' termini.

78. (New) The method of claim 1 or 13, wherein said first and second nucleic acid molecules comprise a topoisomerase recognition site at or near each of their 5' termini.

79. (New) The method of claim 15, wherein said first and second nucleic acid molecules are topoisomerase-charged at or near each of their 5' termini.

80. (New) The method of claim 25, wherein said first and second nucleic acid molecules comprise a topoisomerase recognition site at or near each of their 5' termini.

81. (New) The method of claim 1, wherein said first nucleic acid molecule comprises a topoisomerase recognition site at or near the 3' and 5' termini of one strand, and said second nucleic acid molecule comprises a topoisomerase recognition site at or near the 3' and 5' termini of one strand.

82. (New) The method of claim 15, wherein said first nucleic acid molecule is topoisomerase-charged at or near the 3' and 5' termini of one strand, and said second nucleic acid molecule is topoisomerase charged at or near the 3' and 5' termini of one strand.

83. (New) The method of claim 1, wherein said first nucleic acid molecule comprises a topoisomerase recognition site at or near the 3' terminus of one strand and the 5' terminus of the second strand, and said second nucleic acid molecule comprises a topoisomerase recognition site at or near the 3' terminus of one strand and the 5' terminus of the other strand.

84. (New) The method of claim 15, wherein said first nucleic acid molecule is topoisomerase charged at or near the 3' terminus of one strand and the 5' terminus of the other strand, and said second nucleic acid molecule is topoisomerase-charged at or near the 3' terminus of one strand and the 5' terminus of the other strand.

85. (New) The method of claim 1, further comprising a third linear ds nucleic acid molecule.

86. (New) The method of claim 85, wherein said third ds nucleic acid molecule comprises a topoisomerase recognition site at or near both of its ends.

87. (New) The method of claim 22, wherein the type IB topoisomerase is a poxvirus type IB topoisomerase.

88. (New) The method of claim 27, wherein the type IB topoisomerase is a poxvirus type IB topoisomerase.